DETAILED ACTION

Continued Examination Under 37 CFR 1.114

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submissions filed on January 31, 2008 and March 31, 2008 have been entered. Claims 40-61 are pending and under examination.

Response to Amendment

2. The rejection of claims 40 and 43-50 under 35 U.S.C. 103(a) as being unpatentable over Miyahira *et al*. (*Journal of Immunological Methods*, 1995, 181:45-54, "Miyahira") in view of Hagiwara *et al*. (*AIDS Research and Human Retroviruses*, January 20, 1996, 12(2):127-133, "Hagiwara"), is withdrawn in view of Applicant's amendment. Miyahira does not teach or fairly suggest the use of peptides without APCs. In fact, Miyahira's teachings appear to rely on the use of APCs pulsed with T-cell activating peptides to achieve the result of detecting $CD8^+$ T-cells, whereas the instant invention as amended does not use APCs pulsed with T-cell activating peptides.

Claims Summary

3. The claims are drawn to a method of diagnosing or monitoring infection with an intracellular pathogen wherein peptide-specific effector T-cells are enumerated, comprising:

a. providing a fluid containing fresh T-cells, which have not been cultured *in vitro*, in contact with a surface carrying an immobilized antibody to IFN-γ,
b. presenting to the T-cells a T-cell-activating peptide, wherein the peptide is derived from the intracellular pathogen in the absence of any antigen presenting cells (APCs) pre-cultured with said peptide,
c. incubating the fluid to cause release of said IFN-γ, and
d. detecting released IFN-γ bound to said immobilized antibody to enumerate said peptide-specific effector T-cells.

The incubation is continued for a time to permit IFN-γ release by only those T-cells that have been pre-sensitized *in vivo* to the T-cell-activating peptide and are capable of immediate effector function without the need to effect division/differentiation by in vitro culture in the presence of the T-cell-activating peptide. Specifically, the intracellular pathogen is selected from the group consisting of hepatitis B (HBV), hepatitis C (HCV), *M. tuberculosis*, *P. falciparum*, human immunodeficiency virus (HIV), and influenza virus. Claims 51-58 are directed specifically to the monitoring of *M. tuberculosis*. The T-cell activating peptide is of 7-12 amino acid residues in length. The peptide is added to the T-cell containing fluid, which is recognized by $CD8^+$ T-cells. The peptide is a pre-determined, more specifically, the peptide is the *M. tuberculosis* ESAT-6 peptide. The T-cells are peripheral blood mononuclear cells (PBMCs). Particularly, the T-cells are taken from a patient known to be suffering, or to have suffered from, infection with an intracellular pathogen. The fluid mixture is incubated under non-sterile conditions. The incubation is continued for a time of 4 to 24 hours, more specifically 6 to 16 hours.

Claim Objections

4. Claims 47 and 59 appear to be redundant. Both encompass the embodiment of incubating the fluid sample for a time of 4 to 24 hours. Correction is required.

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. Claims 40-61 are rejected under 35 U.S.C. 103(a) as being unpatentable over Surcel *et al.* (*Immunology*, 1994, 81:171-176, "Surcel"), in view of Sørensen *et al.* (*Infection and Immunity*, 1995, 63(5):17170-1717, "Sørensen"), and Hagiwara *et al.* (*AIDS Research and Human Retroviruses*, January 20, 1996, 12(2):127-133, "Hagiwara").

Surcel discloses Th1/Th2 profiles in tuberculosis, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. "Proliferation and cytokine production profiles by blood mononuclear cells in response to in vitro stimulation with mycobacterial antigens were compared in patients with active tuberculosis and in sensitized healthy people", page 171, abstract. Surcel uses the ELISPOT assay to measure effector T-cells that produce IFN-γ. Surcel's method uses freshly isolated PBMCs from patients with active tuberculosis. The cells are incubated in 96-well plates for 72 hours, in the presence of antigen, before transfer to anti-IFN-γ antibody-coated nitrocellulose-bottomed plates in the presence of a mycobacterial antigen. The cells were then incubated for 20 hours and subsequently enumerated (page 172, second column, last three paragraphs). Surcel is silent on the ESAT-6 mycobacterial antigen.

However, Sørensen discloses the discovery of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*. Sørensen teaches that ESAT-6 is a 6-kDa early

secretory antigenic target. Sørensen discloses that native and recombinant ESAT-6 elicited a high release of IFN-γ from T-cells isolated from memory-immune mice challenged with *M. tuberculosis* (abstract).

It would have been obvious to use ESAT-6 as the activating peptide in Surcel's ELISPOT method. One would have been motivated to use ESAT-6 because it is a T-cell epitope. Surcel's method is aimed at studying the relationships between epitope specificity and T-cell function (page 172, first column, first paragraph). One of ordinary skill in the art would have been motivated to use Sørensen's antigen as the activating antigen in order to understand the relationship between the ESAT-6 specificity and T-cell function. One would have had a reasonable expectation of success based on Sørensen's disclose that ESAT-6 elicited a high release of IFN-γ from T-cells isolated from memory-immune mice challenged with *M. tuberculosis*.

Surcel's measurement of IFN-gamma producing T-cells involves incubation of T-cells in the presence of a T-cell-activating peptide for, what is reasonably deduced from the context of the protocol, 72 hours (page 172, second column, fourth full paragraph). The incubation of T-cells with T-cell activating peptide for 72 hours would allow memory T-cells to proliferate, thus the measurement of IFN-gamma producing T-cells would include both the memory T-cells and effector T-cells. This measurement of both memory and effector T-cells is not the instantly claimed invention's method of measuring only effector T-cells. However, Hagiwara teaches that ELISPOT results are divergent when studying PBMC that have been cultured and stimulated *in vitro*. While Hagiwara's disclosure is directed to cytokine production in HIV patients, the same concept applies to Surcel's ELISPOT. Hagiwara teaches that since the type and amount of

cytokine produced *in vitro* can be altered by the culture conditions employed, inconsistent results from such studies are not unexpected (page 131, first column). Hagiwara chose an alternative strategy, which was to study cells actively secreting cytokines *in vivo* with an incubation time of 6 hours. Hagiwara's technique monitored the pattern of cytokines produced by cells participating in ongoing immune responses in HIV-infected individuals (see Hagiwara, page 128).

It would have been obvious to incorporate Hagiwara's teachings into Surcel's method. Surcel's method is intended for measuring effector T-cells (active tuberculosis versus sensitized healthy controls, see Surcel's abstract). One would have been motivated to use fresh T-cells in Surcel's method in view of Hagiwara's teachings about how the type and amount of cytokine produced *in vitro* can be altered by the culture conditions employed and that inconsistent results from such studies are not unexpected. Given this teaching one of ordinary skill in the art would have been motivated to reduce inconsistent results by using fresh T-cells, rather than the cells used by Surcel that were cultured *in vitro* prior to the ELISPOT assay. One would have had a reasonable expectation of success that the use of fresh T-cells in Surcel's method would have worked because Hagiwara's method uses fresh T-cells in an ELISPOT assay.

With regard to peptides of 7-12 amino acid residues in length (T-cell activating peptides), it would have been obvious to use a peptide of this length. Epitopes are known to be short peptides; methods of determining epitopes are known and routinely practiced. When given a particular protein, the ordinary artisan can readily determine epitopes, both antibody epitopes and T-cell epitopes. It is well within the ability of the ordinary artisan to identify and produce peptides comprising epitopes of interest regardless of their size/length.

With regard to the intracellular pathogen being HIV, Surcel and Sørensen do not teach the diagnosis or monitoring of HIV infection. However, it would have been obvious to diagnosis/monitor HIV infection using Surcel's method. One would have been motivated to do so by the need felt in the world for treating HIV infection, as evidenced by Hagiwara, or any other disease. Given that ELISPOT assays are generally applicable to any desired peptide of interest, one would reasonably expect that T-cells would be activated by T-cell epitopes of HIV, and that levels of IFN-gamma would be detected if present.

With regard to the limitation that the individual has been immunized with a vaccine, the claim (claim 50) does not specify what vaccine is used for immunization. Most individuals in developed countries are immunized for various diseases, including TB (the BCG vaccine). Thus, the samples used by Surcel/Sørensen/Hagiwara are most likely from individuals that have been immunized at some point in their lives prior to sampling.

Therefore, the invention as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made.

Response to Arguments

7. Applicant's arguments have been carefully considered but fail to persuade. Applicant's substantive arguments are primarily directed to the following:

- Applicant argues that Surcel teaches away from the claimed invention because Surcel discloses the incubation of fresh PBMCs for 72 hours prior to the ELISPOT assay. Applicant asserts that this is evidence that confirms that the prior art generally

believed that IFN-γ ELISPOT assays require culturing T-cells in the presence of antigen for long periods.

- In response to Applicant's argument, note that claims 40-46 and 48-58 do not recite any limitations regarding the length of incubation time. With regard to claims 47 and 59-61 which are limited to incubation times of from 4 to 24 hours, or 6 to 16 hours, the Office is aware that the incubation time used by Surcel exceeds 24 hours. As outlined in the rejection of record, this deficiency is noted and thus the Hagiwara reference is relied upon for its teachings regarding the use of fresh cells and an incubation time of 6 hours.

▪ Applicant argues that Sørensen does not teach the use of ESAT-5 in an ELISPOT assay to diagnose or monitor infection by an intracellular pathogen, and that Sørensen fails to provide a specific sequence of an immunodominant peptide.

- In response to Applicant's argument, Sørensen is not relied upon for the teaching relating to the diagnosis or monitoring of infection. Surcel discloses that method by examining Th1/Th2 profiles in tuberculosis patients, based on the proliferation and cytokine response of blood lymphocytes to mycobacterial antigens. Surcel's interest in cytokine response in patients that are actively infected to sensitized is a method of diagnosing infection or monitoring infection. Note that diagnosing infection and monitoring infection are broad terms, not limited to particular embodiments unless otherwise defined in the specification.

- With regard to the lack of a specific sequence of an immunodominant peptide in Sørensen, such a disclosure is not necessary as the claims do not recite a specific sequence of an immunodominant peptide.

- Applicant argues that Hagiwara's use of a mitogen to stimulate T-cells, and the use of cytokine secretion as an indicator of general immune health, teach away from modifying Surcel, alone or in combination with Sørensen.
  - In response to Applicant's argument, Hagiwara is relied upon for the teaching that ELISPOT results are divergent when studying PBMCs that have been cultured and stimulated *in vitro*. While Hagiwara's disclosure is directed to cytokine production in HIV patients, the same concept applies to Surcel's ELISPOT. Hagiwara teaches that since the type and amount of cytokine produced *in vitro* can be altered by the culture conditions employed, inconsistent results from such studies are not unexpected (page 131, first column). Hagiwara chose an alternative strategy, which was to study cells actively secreting cytokines *in vivo* with an incubation time of 6 hours.
- Applicant argues that there is no basis to conclude that an antigen-specific release of IFN-gamma would be possible without either pre-cultured APC cells or long-term *in vitro* culture of the cells in the fluid sample, or both.
  - In response to Applicant's argument, the incubation of Surcel's cells for 6 hours (as suggested by Hagiwara) is expected to activate T cells and produce IFN-gamma. It is noted that Hagiwara does not teach peptide specificity, however, Applicant appears to be arguing the degree of IFN-gamma produced. Even if

more IFN-gamma is produced when using a mitogen than with a specific peptide, IFN-gamma is expected to be produced at detectable levels by the antibodies that bind IFN-gamma. There is no evidence of record that one would expect the complete absence of detectable IFN-gamma secretion if a specific peptide were used and incubated with T-cells for a period of 6 hours. Given antibody specificity, the shorter incubation time (which does not allow memory T cells to be activated) and the likelihood that at least some IFN-gamma will be secreted if there are antigen specific $CD8^+$ T-cells present, one would reasonably predict that detectable levels of IFN-gamma will be observed.

Conclusion

8. No claim is allowed.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stacy B. Chen whose telephone number is 571-272-0896. The

examiner can normally be reached on M-F (7:00-4:30), alternate Fridays off,. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Bruce Campell can be reached on 571-272-0974. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

/Stacy B. Chen/ 4-17-2008
Primary Examiner, TC1600